ANOPTRAL MICROSCOPE - A NEW OPTICAL DEVICE FOR INVESTIGATING OBJECTS WITH WEAK CONTRAST

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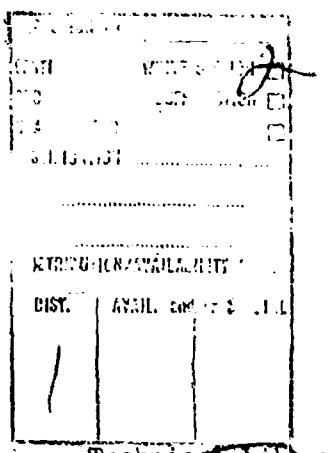

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Following is the translation of an article by M. A. Peshkov, appearing in the Russian-language periodical Uspekhi Sovremennoy Biologii (Successes of Modern Biology) 1955, Vol. 39, Issue 2, pages 253--256. Translation performed by Sp/7 Charles T. Ostertag, Jr.

Since the development of the methods of contrast microscopy (Kohler and Loos, 1941) and the implantation in research laboratories of this valuable method, mastered by our industry a long time ago, more than once attempts were made to improve the work of the phase-contrast microscope which nevertheless possessed many deficiencies. One of the most important deficiencies is the loss of contrast of the image when superimposing one object on another, and also a lowering of resolving power due to a sharp reduction of the numerical aperture of strong objects as a result of the small diameter of the ring-like phase plate and the corresponding reduction of the numerical aperture of the condenser.

An interesting novelty in the field of interference contrasting was the new method of light microscopy suggested by Wilska in 1953. By placing a ring-like layer of soot (analogous to the phase ring of phase-contrast objectives) on one of the objective lenses and using the ring-like diaphragm of the corresponding aperture, fixed in the condenser, Wilska designed a device, giving the effect of negative phase-contrast, but considerably surpassing it and devoid of the above listed defects of this method. The background of the field of vision is an agreeable sepia tone, the contrast of objects is increased extremely and the objects themselves are colored in various shades of a golden-brown tone.

In 1954 a lengthy article by Wilska appeared, where much data was presented concerning the arrangement of a microscope of this type which he named anoptral. Also numerous micrographs were published, illustrating the exceptional properties of the new method.

Taking into consideration the sparse directions of Wilska (1953, 1954) concerning the alteration of ordinary objectives into anoptral (Wilska, 1953, 1954), in August 1954 we were able to prepare our own anoptral microscope and with the help of it conduct several scientific investigations, the results of which were reported: 16 September 1954 at a colloquium of the Cytology Department of the IMZh Institut Morfologii Zhivotnykh A. N. Severtseva, A. N. Severtseva Institute of Animal Morphology (with a demonstration of diapositives), 28 October 1954 -- at the Scientific Council of the IMZh (with a demonstration of diapositives and movie film), on 10 December 1954 at the Histology Section of the Moscow Society of Nature Investigators (also with a demonstration of

diapositives and new film), and on 24 December 1954 at the Leningrad Optical Institute at a colloquium of the A. N. Zakharyevskiy corresponding member laboratory, AN, USSR. Reports about the results of working with this new device caused considerable interest everywhere. This makes it proper to strongly recommend the anoptral microscope to the attention not only of microscopist--cytologists, but to all other persons of other specialities which use the microscope to study objects of weak contrast. Our optical industry must be convinced of the necessity for the most rapid mastering of and production of anoptral microscopes for wide utilization. This will increase the power of our scientific armament with new methods or research.

For preparing the anoptral microscope, we used a Reichert screw-type objective -- homogeneous oil immersion 1/12, inherent magnification 100. The objective was screwed off. On the upper surface of the penultimate lens (counting from the front one) a layer of soot is placed. It is obtained by the burning of dimethylbenzene. The layer should be of such a thickness that it permits the passage of only 10% of passing light (the latter is determined empirically). Then the body of the objective, which has been screwed together, is fastened in the chuck of a lathe, and at a speed that is quite high the layer of soot is ground off from the edges of the lens with a copper needle. Then they select the center of the small circle being formed so that a ring of soot is obtained, the diameter of which should somewhat exceed half the diameter of the exit pupil of the converted objective. The width of the ring is established empirically. It is natural that the center of the ring should coincide with the optical axis of the objective; this is achieved by the correct centering of the objective when fixing it in the chuck of the lathe.

After preparing the ring its dimensions are verified on the assembled objective which is set up on the microscope and focused on the object, after which the ocular is replaced by the auxiliary microscope from the phase-contrast device. Then a glass disc is prepared which should freely enter underneath into the condenser mounting for the aplanatic objective, the numerical aperture of which equals 1.4. A small circle made of black paper is pasted on the center of the disc. The diameter of the small circle is adjusted empirically, observing its image with the help of the auxiliary microscope so that the cross section of its profile in the exit pupil of the anoptral objective was precisely equal to the inner diameter of the ring made from the layer of soot. The disc carrying the small black circle is moved into the condenser mounting where it is clamped with a special spring. The clearance in the disc is used for centering the small circle in relation to the ring of soot, which is done under the control of the auxiliary microscope. Upon completion of centering, the glass disc is tightened by the aforementioned spring.

The Path of Light Rays in an Anoptral Microscope

The lower black circle a depicts the vertical projection of the ring diaphragm a of the condenser b. Light, falling through the ring diaphragm a, is focused by condenser b on the preparation c and, passing through the object, reaches the objective d. In the exit pupil of the objective d, on surface e there emerges an image of the ring diaphragm a on which is superimposed the semitransparent ring of soot f, located between the upper lenses of the objective; the vertical projection of this ring f is depicted over the objective. In this manner, the amplitude of nondiffracted light during passage through the layer of soot of the ring f is reduced by 90%, while the amplitude of diffracted rays is not changed. Furthermore, the ring-like film of soot which includes, along with the carbon, products of the incomplete burning of dimethylbenzene, apparently acts as an inhibiting phase plate so that the rays of the neutral maximum undergo not only a reduction of amplitude, but also a negative phase shift, which after interference with the diffracted rays on the surface of the image of the object also leads to the appearance of an anoptral image. Furthermore, the film of soot acts as a thick light filter, lowering the chromatic aberration of the objective and causing the coloration of the background and images of the objects to be an agreeable sepia tone.

The iris diaphragm of the Abbe illuminating apparatus of the microscope itself is used as the outer edge of the ring-like diaphragm. This is preferable in comparison to Wilska's method since it makes it possible to regulate the effective width of the ring of soot and by this it considerably changes the degree of contrast of the anoptral image.

For setting up the lower ring diaphragm it is possible to use the centering adjustment of the phase-contrast unit, having substituted in it a condenser for high aperture (1.4) and having placed in the free openings of the diaphragms the small glass circles with special high aperture ring diaphragms, adjusted to the objectives which had been made.

Nevertheless, the first unit permitting the regulation of the width of the soot ring is undeniably advantageous and this cannot be ignored.

For illumination of the object in an anoptral microscope the Kohler method (1893) is used. This is described in detail in the instruction book for the phase-contrast unit which is included with each device (see also Shillyaber, 1951).

Sequence of Work with the Anoptral Microscope

The object is mounted on a slide in a Sh-shaped chamber based on Peshkov (1948) or in a Fonbrune oil chamber (1950), with the consideration that the overall thickness of the preparation does not exceed 1.2 mm,

since otherwise it would not be possible to realize the precision illumination according to Kohler.

Illumination was established according to Kohler with a slight magnification. This was done by removing the condenser ring diaphragm and obtaining in the center of the field of vision both a sharp image of the object and of the small luminous circle, circumscribed by the edges of the diaphragm of the field of the lamp collector which had been contracted as much as possible. Then the anoptral objective is set up. It is focused on the object and, having replaced the ocular with the auxiliary microscope, under visual control the ring-like diaphragm of the condenser (or only its central part -- the small black circle) is centered in relation to the ring of soot. The auxiliary microscope is replaced by the ocular and the study of the preparation commences.

At the same time that the field of vision of a phase-contrast-microscope is illuminated 30 times weaker than the field of vision of an amplitude microscope with an alalogous objective and the same ocular, in an anoptral microscope, as a result of the passage by the ring of soot of all told 10% of the light, the illumination is lowered yet by two times. This, however, does not serve as an obstacle for obtaining micrographs both with a small exposure (10--20 sec) as well as of instant photos (speed filming) when using a low voltage 50 watt tube as the source of light and a strong light commutator.

As Wilska already pointed out, the pictures observed in an anoptral microscope are considerably superior in contrast than those usually seen with the phase-contrast method. The unusually agreeable, warm brown tone of the background, the very unusual body of the image of objects, the opportunity to observe multilayer objects without any loss of their contrast, the plasticity and at the same time sharpness of outline of objects create a very special condition in the observer, whose acuteness of vision is undoubtedly increased by these conditions. What has been said is illustrated by the accompanying microphotographs.

Microcolonies of the bacteria Caryophanon latum are depicted on the microphotographs (figures 2, 3). The light cell membranes and numerous cell walls of this multicellular bacteria stand out very sharply. Appearing in exceptional relief is the form of individual cells and their relative arrangement in respect to each other, which is usually not exposed by other microscopic methods. Individual specimens appear illuminated as if from within. One microphotograph (figure 3) depicts the edge of an analogous microcolony of Caryophanon latum, incubated on a thin layer of agar in an oil chamber. The specimens are unusual in relief and call to mind tangles of coiling snakes. Another microphotograph (figure 4) shows four microcolonies of Bac. megatherium, also incubated in an oil chamber. Clearly discernable are the cell membranes, large fatty inclusions in the form of light globules and the

finest inclusions in the protoplasm of specimens, which themselves prove to be made up of lighter (dense) and darker (less dense) sectors. The microphotograph (figure 5) has grasped the multiplication of sporogenous bacilli. Also visible in the specimens are cell membranes, cell walls, inclusions of protoplasm and several details of its structure. The next microphotograph (figure 6) is a print of a live smear of fish blood (macropod). Erythrocytes and white elements slipping off into the porous system are visible. The depressed form of the erythrocytes and their darker structurized nuclei are clearly noticeable. The structural details of the sliding leucocytes is very interesting. Attention is called to the extreme voluminosity of the group of leucocytes, which seems as if stuck together.

When studying the image of objects in an anoptral microscope the impression is created that in the field of vision there are zones of dark and light situated over each other; a volumetric object seems partially immersed in both zones, which furthers the extreme relief of the image. It is understood that this is only an impression, at the basis of which lies a yet unexplained physical phenomenon. Personnel acquainted with the Fuko shadow method which is used for the investigation of forms of optical surfaces, when working with the anoptral microscope cannot get rid of the sensation that in this case the increased relief of the image is somehow connected with the knife principle (shadow method), the role of which may be played by the edge of the ring-like diaphragm, but 90% of the nondiffracted light is absorbed by the soot ring, as a result of which a sufficiently contrasting background is created for obtaining one of the effects of dark field, on which the shadow method is based.

Inasmuch as even on the problem concerning the mechanism of image formation in the phase-contrast microscope not everything is completely clear (Wilska, 1953, 1954), it is more difficult yet to establish to what category of optical phenomena the pictures appearing in an anoptral microscope should be regarded. If Wilska himself (1953) does not go into a discussion of this interesting problem, only contrasting the pictures given by the anoptral microscope with the image of a positive phase-contrast device, then Barer (1953) in his article "New Method of Light Microscopy" points to the fact that the anoptral method is based on the use of the principle of "amplitude contrast" (Oettle, 1950), first observed already by Bratuscheck in 1892. This method is based on the artificial absorption of a considerable amount of nondiffracted light, which is achieved by mounting in the exit pupil of the objective an absorbing layer, screening off the area of the neutral maximum. During this, the amplitude of light, diffracted by the amplitude object, is not lowered. As a result of the subsequent interference of diffracted and weakened nondiffracted rays in the plane of the object's image, its image emerges considerably more contrasting than the object

itself (see Oattle, 1950). With complete opacity of the ring of soot, an image would emerge which is constructed exclusively by means of diffracted rays, that is, formed on the principle of dark field with a central darkening in the objective. Barer (1950) also dwelled on the possibility of the emergence of a phase effect, caused by the layer of soot, which probably works as an inhibiting film, guaranteeing the emergence of negative phase-contrast superimposed on the effect of amplitude contrast. Apparently Wilska himself (1953a) agreed with these findings. The dark fringe surrounding the object (see microphotographs, figures 1--5) also speaks in favor of the phase-contrast nature of the anoptral image. This, in the opinion of Zernike (1942) emerges as a result of the interference of diffracted rays, partially penetrating through the phase plate, while the width of the fringe is usually reversely proportional to the width of the phase ring.

In this manner, apparently, the anoptral microscope is a variety of the amplitude and negative phase-contrast microscope, but the possibility is not excluded of the emergence of pictures which are observed in the Fuko shadow method (knife method). Without devoting ourselves further on the theoretical foundation of the method, which it is better to assign to our professional opticians, it can be confirmed again that there is considerable advantage in the anoptral microscope, and in particular in the extremely great depth of the field of the object, which in an immersion system reaches a value of 2 μ (in the ordinary immersion 90 the depth of the field does not exceed 0.4 μ). (Shillyaber, 1951). Having justified for practical use the excellent results obtained during time lag motion picture taking of delicate viable processes such as the division of cell nuclei of Protozoa, and the detection of the most minute details of protoplast, emerging during the division of bacteria, there is cause to express again the desire for the speedy mastering of the production of this simple device by our optical industry and distributing it for sale for general use.

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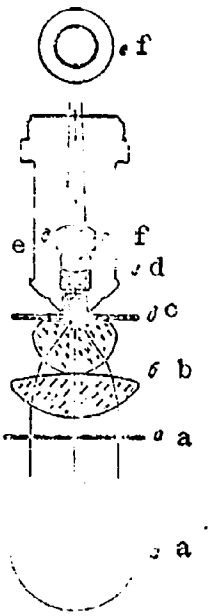

Figure 1.

a -- lower black circle
a -- ring diaphragm
b -- condenser
c -- preparation
d -- objective
e -- surface
f -- semitransparent ring of soot

GRAPHIC NOT REPRODUCIBLE

Figure 2. Caryophanon latum. Oil chamber. The multicellular texture of the species is visible. The membrane and cell walls are light. The protoplasm is dark. X 3000.

Figure 3. Caryophanon latum. Oil chamber. Edge of the colony. The prominence of the image is stressed. X 3000.

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Figure 4. Bac. megatherium. Microcolonies. Oil chamber. The internal structure of the specimens is visible. X 3000.

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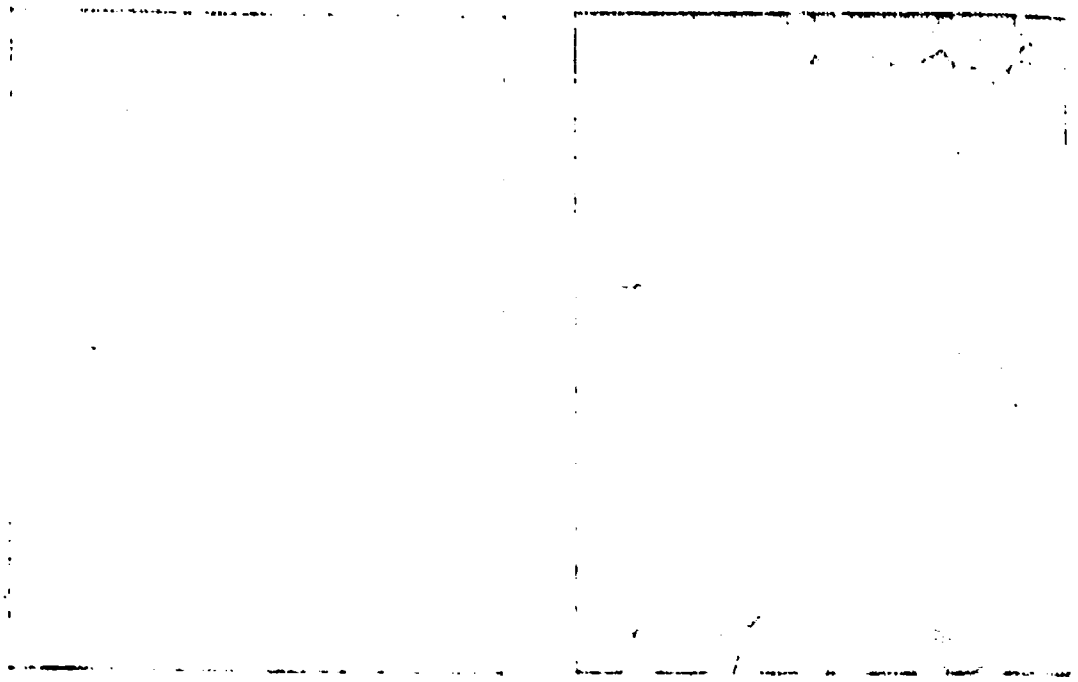

Figure 5. Bac. sp. Growth of microcolony. The structure of the specimens and the cell walls are visible. Oil chamber. X 3000.

Figure 6. Blood of a macropod. Live preparation. The white elements are slipping off into the porous system. The flat form of the erythrocytes and their darker nuclei are visible. X 2000.